Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary information

Coproduction of succinic acid and cadaverine using lysine as neutralizer and CO_2 donor with L-lysine decarboxylase overexpressed *Escherichia coli* AFP111

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Supplementary Methods and Results

Calculation of CO_2 net emission

To verify the net emission of CO_2, a contrastive experiment with a different reactor and small system was conducted. After cell cultivation and decarboxylase induction, the fermentation broth supplemented with glucose (10 g L^{-1}) was transferred to a sealed and sterilized blue-lid flask (10-mL) with N_2 access for 2 min to ensure anaerobic conditions. The contrastive experiment was performed with supply of 10 g L^{-1} lysine to utilize decarboxylate CO_2. At the end of anaerobic fermentation, concentration of succinic acid, cadaverine, acetate and ethanol was investigated to calculate the net emission of CO_2. Experiments were carried out in triplicate, and the data are presented as mean values with standard deviation.

Investigation of aspartate and amino acids derivatives

The aspartate metabolic pathway was shown as Fig. S5. Aspartate and some of amino acid derivatives (marked in red) in the same samples were also analyzed by HPLC. The exact product concentrations were determined using high-performance liquid chromatography after labelling with phenylisothiocyanate (PITC), using a 1290 facility (Agilent Technologies, USA) equipped with a C18 column (5 μm, 250 mm × 4.6 mm, Grace, USA) at room temperature. Mobile phase A consisted of 7% (v/v) acetonitrile in 0.1 M sodium acetate aqueous solution, and mobile phase B consisted of 80% (v/v) acetonitrile in water. PITC derivatives were separated with a gradient of 97:3 to 30:70 (v/v) of A: B over 50 min and detected at an absorbance of 254 nm. The results were list in Table S1. Furthermore, the important metabolite aspartate was also detected by MS facility and the results suggested that only about one third of it was ^{13}C labelled aspartate (Fig. S6). Experiments were carried out in triplicate, and the data are presented as mean values with standard deviation.

Calculation of CO_2 fixation efficiency by carboxylation metabolic pathway of succinic acid

To calculate amount of CO_2 released from decarboxylation process using for the carboxylation metabolic pathway of succinic acid. The contrastive experiment of succinic acid fermentation was performed without supply lysine as CO_2 donor as

control. The amount of decarboxylate CO_2 utilized by the carboxylation metabolic pathway of succinic acid (E) can be calculated through the following equation:

$$E = \frac{F_1 - F_0}{R} \times 100\%$$

here

F_1, represents the amount of fixed CO_2 by the succinic acid pathway with the supply of L-lysine, which can be calculated form succinic acid produced.

F_0, represents the amount of fixed CO_2 by the succinic acid pathway without the supply of L-lysine, which can be calculated form succinic acid produced.

R is the amount of CO_2 released from decarboxylation process, which can be calculated form cadaverine content.

With the supply of L-lysine, succinic acid was detected as 0.18 mol L^{-1}, while the production of succinic acid without any supply of L-lysine was 0.014 mol L^{-1}. Furthermore, the amount of CO_2 released from decarboxylation process was detected as 0.22 mol L^{-1}. Thus, the final equation for calculation of the CO_2 fixation efficiency is as follows:

$$E = \frac{0.18 - 0.014}{0.22} \times 100\%$$

These results showed that 75.45% of CO_2 released from decarboxylation process could be utilized by the carboxylation metabolic pathway of succinic acid.

Supplementary Tables

Table S1 Concentrations of amino acids involved in aspartate metabolism

Amino acids	Concentrations (mmol L^{-1})
Aspartate	2
Methionine	0.078
Arginine	0.067
Citrulline	0.047
lysine	Not detected
ornithine	Not detected

threonine	Not detected

Supplementary Figures

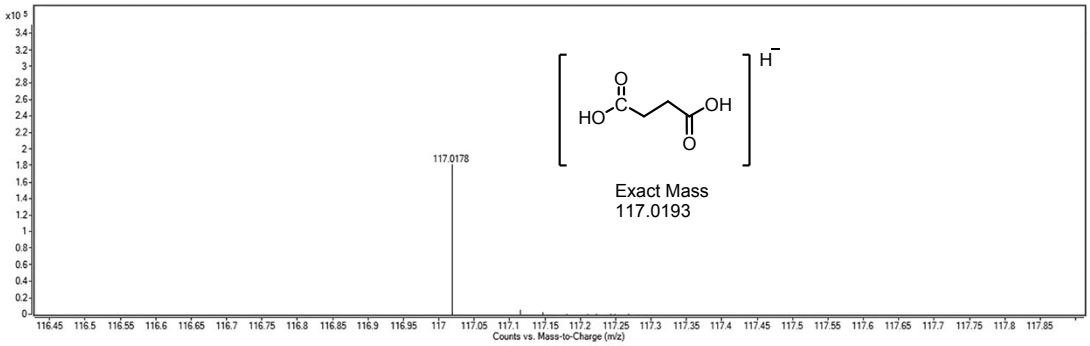

Figure S1 ESI-TOF-MS spectrum of ^{12}C-Succinic acid

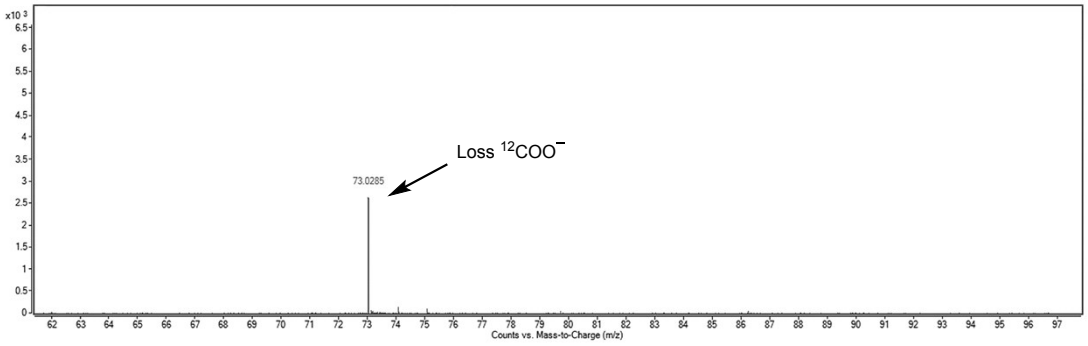

Figure S2 ESI-TOF-MS/MS spectrum of ^{12}C-Succinic acid

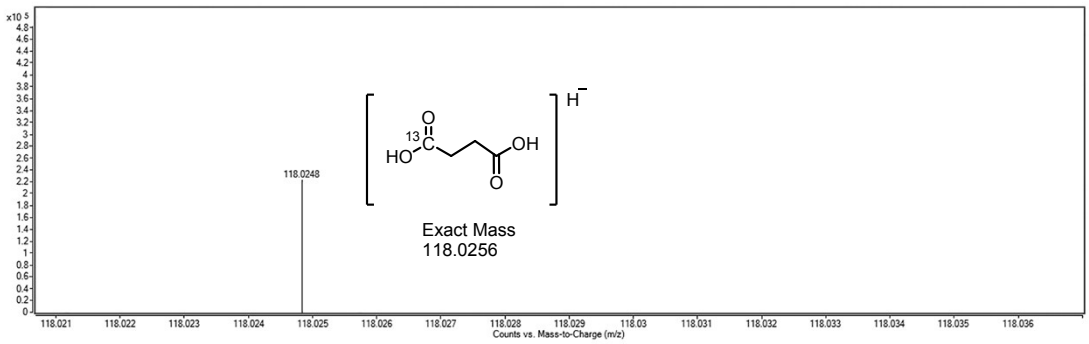

Figure S3 ESI-TOF-MS spectrum of ^{13}C-Succinic acid

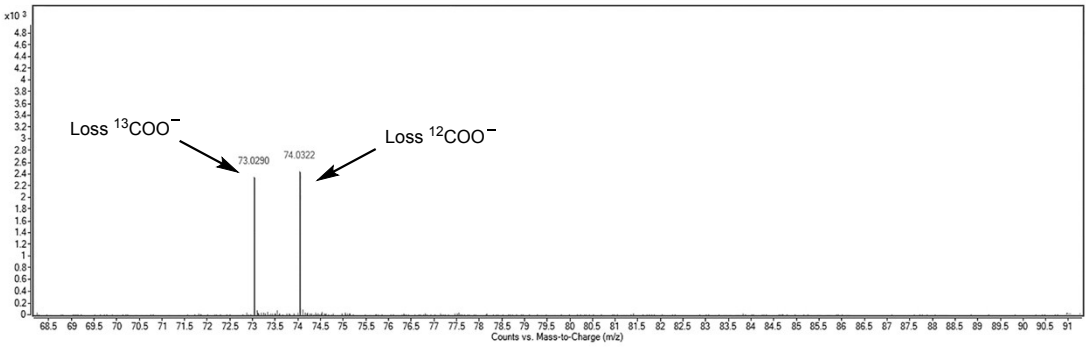

Figure S4 ESI-TOF-MS/MS spectrum of ^{13}C-Succinic acid

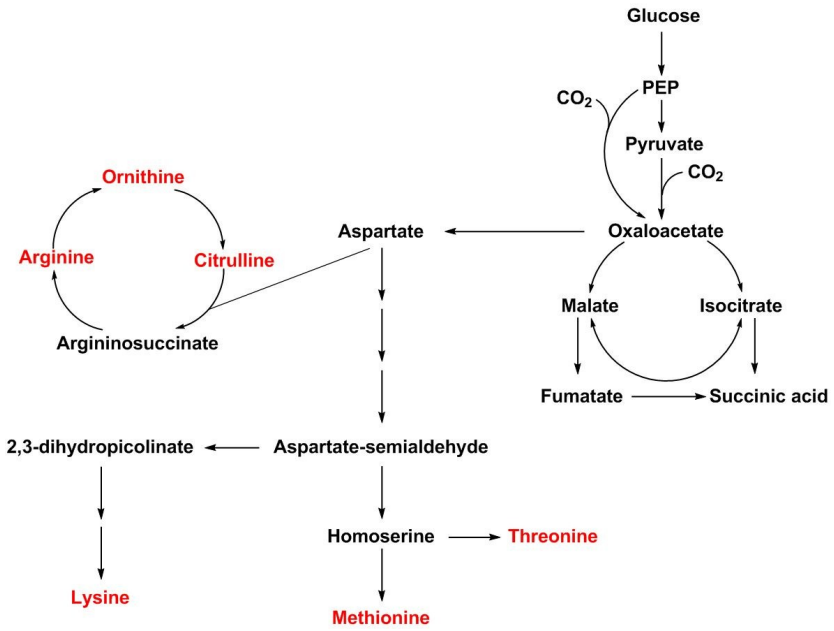

Figure S5 Brief biochemical pathways of aspartate derivatives in *E. coli*

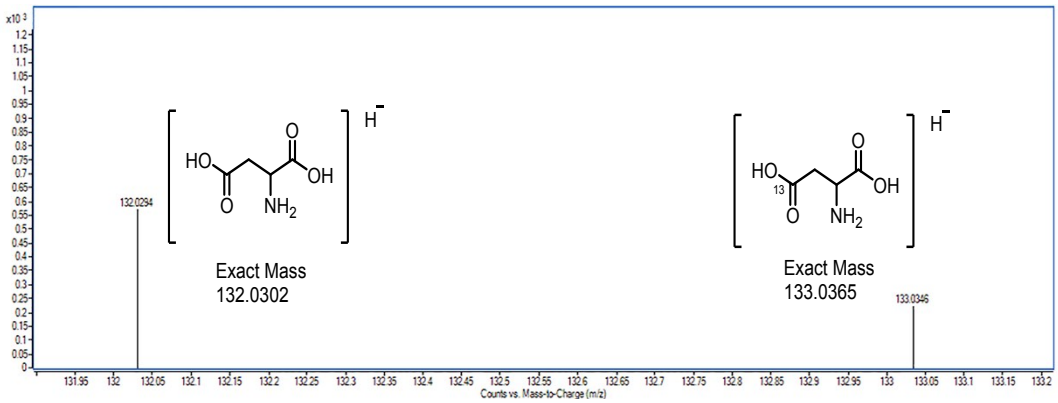

Figure S6 ESI-TOF-MS spectrum of ^{12}C-Aspartate and ^{13}C-Aspartate

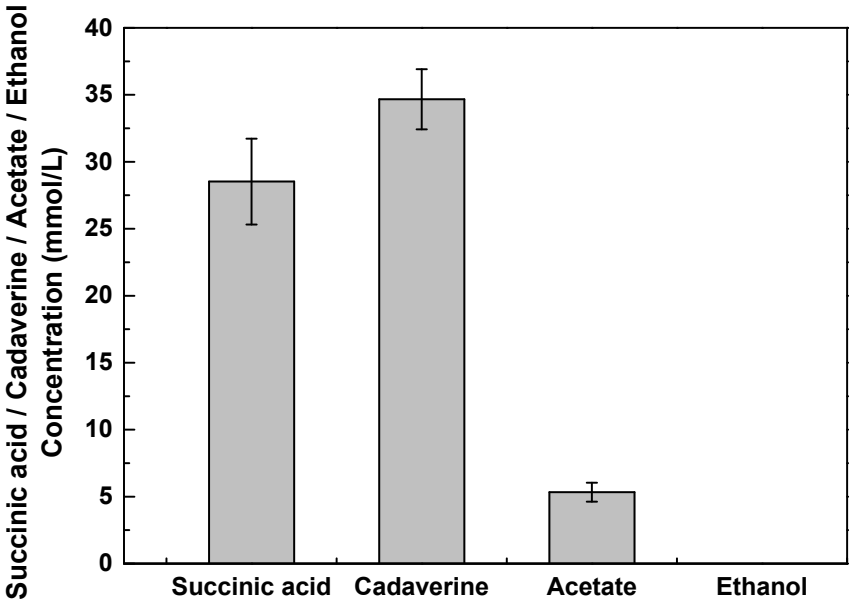

Figure S7 Net emission of CO_2 during anaerobic stage

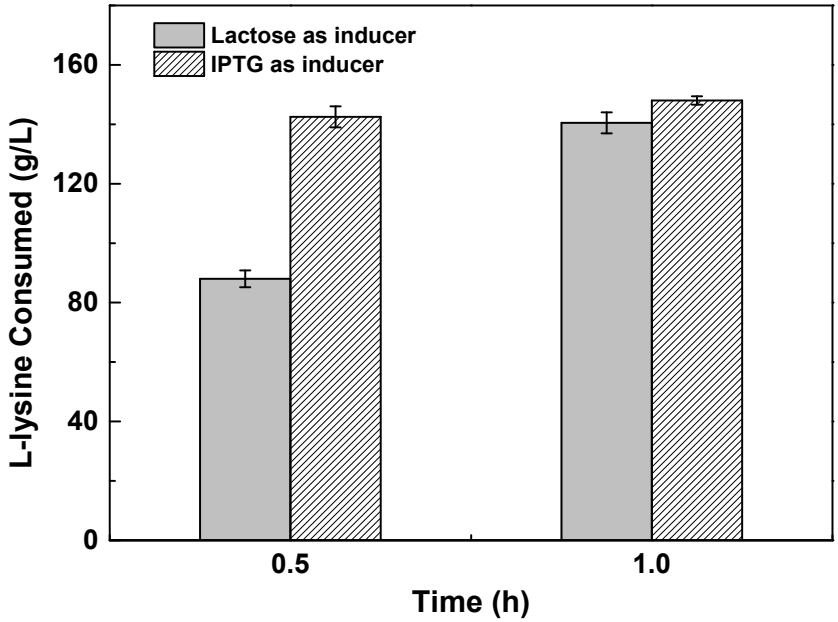

Figure S8 The effects of different inducers on enzyme activity of CadA by analyzing L-lysine consumption